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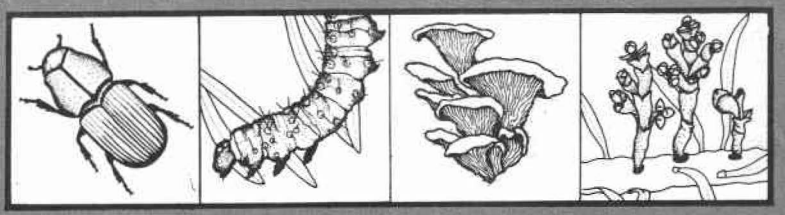

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EPIDEMIOLOGY OF *FUSARIUM* ON CONTAINERIZED DOUGLAS-FIR SEEDLINGS. 1. SEED AND SEEDLING INFECTION, SYMPTOM PRODUCTION, AND DISEASE PROGRESSION

by

R. L. James, Plant Pathologist 1/
R. K. Dumroese, Research Associate 2/
D. L. Wenny, Associate Professor 2/
J. F. Myers, Nursery Superintendent 3/
C. J. Gilligan, Biological Technician 1/

ABSTRACT

Investigations were conducted to understand the epidemiology of *Fusarium* on containerized Douglas-fir seedlings. Types and importance of *Fusarium* inoculum sources, relationships between seedling infection and symptom production, amounts and types of diseases that occurred throughout typical growth cycles, and the importance of secondary pathogen spread were investigated. Levels of *Fusarium* on seed could not be used to accurately predict disease incidence within a seedlot. Inverse correlations existed between the amount of *Fusarium* and *Trichoderma* on seed. Higher levels of *Fusarium* were detected on bleach-treated seed, probably because of reductions of *Trichoderma* populations on treated seed. *Fusarium* commonly colonized seedling roots without causing disease symptoms. Investigations failed to show spread of *Fusarium* disease from one container to another. Factors affecting disease symptom expression and implications for control are discussed.

1/ Timber, Cooperative Forestry and Pest Management.

2/ College of Forestry, Wildlife and Range Sciences, University of Idaho, Moscow.

3/ USDA Forest Service Nursery, Coeur d'Alene, Idaho.

United States Department of Agriculture | Forest Service | Northern Region | P.O. Box 7669 Missoula, Montana 59807

INTRODUCTION

Fusarium spp. cause important diseases of conifer seedlings at nurseries in western North America. These fungi cause damage to many conifer species, but diseases of containerized Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings are especially important because extensive losses often occur (James 1985b; James and Gilligan 1987). Major diseases caused by *Fusarium* on containerized Douglas-fir seedlings include pre- and post-emergence damping-off during and shortly after germination and root disease on older seedlings. *Fusarium* spp. are common colonizers of conifer seed (James 1984; James 1986a; James 1986b) which may serve as an important inoculum source in container operations. Losses from *Fusarium* diseases vary with each crop but most nurseries in the northern Rocky Mountains sustain some level of damage each year. Seedling mortality was previously thought to be the major impact of these diseases. However, other effects including growth reduction and reduced seedling vigor may be equally important.

A major problem confronting growers who deal with *Fusarium* diseases is the difficulty of control. Damping-off may be relatively easy to control with fungicides. However, root diseases of older seedlings are much harder to control because fungicide drenches are usually not effective. Although losses continue to occur, very little is known about the basic epidemiological behavior of *Fusarium* on containerized conifer seedlings. Therefore, investigations were initiated to help understand the epidemiology of *Fusarium* on containerized Douglas-fir seedlings in order to formulate more effective control procedures. Studies were specifically designed to evaluate types and importance of inoculum sources, relationships between seedling infection and symptom production, amounts and types of diseases that occur throughout typical growth cycles, relationships between disease occurrence and cultural practices, and importance of secondary pathogen spread. A major goal of this work was to determine if levels of seedling infection and disease could be predicted from the amount of *Fusarium* on seed. If so, relatively simple seed assays could provide growers with valuable information regarding disease potential in their crop. With such information, growers would be able to direct control efforts where they would be most effective. Future reports will address characteristics and pathogenicity of some isolates of *Fusarium* associated with certain types of diseases and evaluation of the fate of asymptomatic seedlings with *Fusarium* root infection after outplanting.

MATERIALS AND METHODS

Study Sites

Two nurseries in northern Idaho were selected as sites for these studies: the USDA Forest Service Nursery, Coeur d'Alene, and the University of Idaho Research Nursery, Moscow. Both nurseries have a history of moderate damage from *Fusarium* diseases on their containerized Douglas-fir, and are considered average for Northern Rocky Mountain nurseries.

Seed Infection

Previous investigations (James 1985a; James 1986a) have implicated seed as a major source of *Fusarium* inoculum in containerized operations. Therefore, representative seedlots were selected from each nursery (Table 1 - Appendix) to be evaluated for levels of seed infection and subsequent disease development. The five seedlots selected from the USDA Forest Service Nursery were representative of Douglas-fir lots at the lowest level of accepted germination. These lots were expected to have slightly greater than normal problems with germination and somewhat higher disease levels. The three seedlots selected from the University of Idaho Research Nursery were common lots that had been used for several years; all three seedlots had germination rates at acceptable levels.

Seed from the University of Idaho Research Nursery was operationally treated with aqueous sodium hypochlorite (commercial bleach) prior to sowing to improve seedling survival and reduce damping-off losses (Wenny and Dumroese 1987). The treatment consisted of filling mesh bags with seed and immersing the filled bags for 10 minutes in a solution of 40 percent commercial bleach and 60 percent water. Following treatment,

seed was rinsed in tap water to remove residual bleach and placed in a running water rinse for 48 hours. After rinsing, seed were stratified for 21 days at 0.5-3.3 degrees C and rinsed once more for 24 hours prior to sowing.

--USDA Forest Service Nursery

At the time of sowing, five trays (each containing 200 small Leach® pine cells) were randomly selected from each seedlot and designated "study trays." Twenty cells per study tray were randomly selected for seed sampling. One of the seeds sown in each selected cell was collected and assayed for *Fusarium*. Half of the seed thus collected were aseptically dissected to evaluate occurrence of *Fusarium* within the endosperm and on the seedcoat.

Samples of 200 seeds from each of the five seedlots were randomly collected from bulk seed and assayed for presence of *Fusarium* and other fungi on their seedcoats. From bulk seed, 20 pieces of seed debris (cone scales, wings, pieces of organic matter, resin globules, etc.) were also randomly selected and assayed for presence of *Fusarium*.

Germination was estimated from a sample of 250 randomly selected seed. Seed were selected from bulk and **not** preconditioned (soaked in water) prior to germination testing. Selected seed were placed on 2 percent water agar in a growth chamber with a 12-hour diurnal light and darkness cycle at about 22 degrees C. Germination was determined after 28 days' incubation. Germination rates in this test were compared with standard tests conducted by the Nursery.

All seed assays and isolations from seedlings were placed on a selective medium for *Fusarium* (Komada 1975). Plates were incubated under cool fluorescent light at about 22 degrees C for 7 days after which they were examined for presence of *Fusarium* and other fungi.

--University of Idaho Research Nursery

Bleach-treated and untreated seed from each of the three seedlots were compared for presence of *Fusarium* and other fungi. Each sample consisted of 200 seed randomly selected for each treatment per seedlot. Fifty seeds were also aseptically dissected to determine presence of *Fusarium* within seed endosperms and on seedcoats.

Germination tests for each of the three seedlots were conducted within greenhouses. Seven hundred ninety-two seeds per seedlot were assayed for germination on standard container soil mix. Germination was determined after 28 days' incubation.

Damping-off

--USDA Forest Service Nursery

For each study tray, number of emerged germlings per cell were counted when germination was complete (just prior to thinning). At that time, amount of post-emergence damping-off was determined and germlings affected were collected for isolation of associated Fusaria. Twenty cells were randomly selected from each study tray; within each of these cells all germlings that would have been thinned (one emerged germling left per cell) and lacked disease symptoms were collected for determining presence of *Fusarium* spp. on their main root. Selected germling roots were incubated on the selective medium and the number of *Fusarium* colonies per unit length of root determined. Within these cells all ungerminated seed were also collected for assay for presence of *Fusarium*. After thinning, the number of empty cells for each seedlot was estimated by sampling a representative number of trays.

--University of Idaho Research Nursery

For each seedlot, two study trays (styroblock 4A's, each containing 198 cells) were randomly selected from both bleach-treated and untreated seed. For each study tray, post-emergence damping-off was determined at the time of thinning. Asymptomatic germlings were randomly collected from 20 cells from each study tray for assay of *Fusarium* on their roots as described above.

Root Diseases

At each nursery the study trays were periodically examined for seedlings with disease symptoms. All diseased seedlings were collected and isolations made from their roots for presence of *Fusarium*. Since there were often few diseased seedlings in the study trays, root-diseased seedlings were often selected from throughout the sampled seedlots. Extent of root colonization was determined by aseptically placing sections of 10 lateral roots from each seedling on the selective medium. Percentage root system colonization was then calculated by determining the number of root pieces colonized.

At the USDA Forest Service Nursery, periodic collections of weeds from greenhouse floors were made throughout the growth cycle and assayed for the presence of *Fusarium.*

Asymptomatic Seedling Infection

At the USDA Forest Service Nursery, four samples of asymptomatic seedlings were collected for determining presence and extent of *Fusarium* on their root systems. The first sample was taken at the time of thinning as described above in the damping-off section. All other samples were from 10 randomly selected seedlings from study trays; sampling occurred at approximately monthly intervals.

At the University of Idaho Research Nursery, five samples of asymptomatic seedlings were collected. Likewise, the first sample was at the time of thinning. Other samples occurred at 1- or 2-month intervals except the last sample which occurred during seedling lifting.

Root isolations from asymptomatic seedlings were similar to those described above for root-diseased seedlings. Number of seedlings infected and percentage root system colonization by *Fusarium* were determined.

Secondary Disease Spread

At the USDA Forest Service Nursery, 25 diseased seedlings from throughout the seedlot, but not located within the study trays, were selected as possible foci for secondary spread of *Fusarium*. Presence of sporodochia on the diseased seedlings above the groundline was the major criterion for selection. Selected seedlings were marked and adjacent seedlings examined periodically throughout the growing season for presence of disease symptoms.

Data Analysis

Data were analyzed using a one-way or two-way analysis of variance, Tukey's comparison test of means, a standard "t" test to determine significant mean differences, and simple regressions (either linear or logarithmic) to evaluate correlations between sets of data.

RESULTS

Seed Infection

Occurrences of *Fusarium* and two other common seed-colonizing fungi (*Trichoderma* and *Penicillium*) on Douglas-fir seed are summarized in Table 2 (Appendix). On seedlots from the USDA Forest Service Nursery, there were significant (P=0.05) differences in occurrence of all three fungal genera among the five seedlots tested. Percentage infection with *Fusarium* ranged from 1.3 to 15.0; the average for all tested seedlots was 8.1 percent. Extent of *Fusarium* assayed within endosperms was more consistent among the seedlots tested and ranged from 2 to 8 percent. Colonization of seed debris with *Fusarium* was generally higher than that found on seedcoats, but the amount sampled was much less. For the University of Idaho Research Nursery, occurrence of these fungi on seed also varied among the seedlots tested. Standard bleach treatments significantly (P=0.01) reduced occurrence of *Trichoderma* on seedcoats, but levels of *Fusarium* spp. were consistently detected at higher levels on bleach-treated seed. Effects of bleach treatments on seed populations of *Penicillium* varied among the different seedlots, but were not significant when all seedlots were compared together.

Germination test results for the sampled seedlots are summarized in Table 3 (Appendix). The relatively low germination rates obtained for the five seedlots from the USDA Forest Service Nursery may have been due to lack of preconditioning seed (such as a water soak) prior to testing and because the test was conducted on water agar instead of either soil mix (as was done for the University of Idaho seedlots) or in standard germination test containers. In any event, germination results obtained on water agar did not correspond closely to reported germination rates from the Nursery. For seedlots tested at the University of Idaho Research Nursery, treatment of seed with bleach significantly (P=0.05) improved germination of only one seedlot (FN); however, when all seedlots were combined, treatment effects were significant.

Comparisons among fungi on seed and seed germination are summarized in Table 4 (Appendix). Simple linear regressions were used initially to compare variables; if high coefficients of determination (R2) were obtained, other equations (such as logarithmic, exponential, and power) were tested to best fit the data. Values included in Table 4 represent the highest correlations between variables. Occurrence of *Fusarium* on seed was not highly correlated with germination at either nursery (equations 1 and 6), although *Fusarium* within the endosperm was more correlated with germination (equation 2). However, occurrence of *Trichoderma* seemed to be related to germination (equations 3 and 7), particularly at the USDA Forest Service Nursery. Occurrence of *Fusarium* on seed was inversely related to amounts of *Trichoderma* on seed at the University of Idaho Research Nursery (equation 8); however, the same relationship was not evident at the USDA Forest Service Nursery (equation 4). Also, there were strong correlations between the occurrence of *Trichoderma* and *Penicillium* on seed at both nurseries (equations 5 and 9).

Disease Incidence

For purposes of this paper, "infection" refers to colonization of seedlings by *Fusarium* with or without above-ground symptom production. However, "disease" refers to the conditions of symptom production (tissue chlorosis and necrosis) on infected seedlings.

Occurrence of *Fusarium*-related seedling diseases was very low throughout the growth cycle at both nurseries. Data on disease incidence were more complete and meaningful at the USDA Forest Service Nursery because of larger sample sizes. Only a couple of diseased seedlings were found in the study trays from the University of Idaho Research Nursery. Table 5 (Apprendix) summarizes data on disease incidence at different times throughout the growth cycle at the USDA Forest Service Nursery. Significantly more disease occurred during the first month of the growth cycle than at other times. Early disease was primarily pre- and post-emergence damping-off and cotyledon blight. Diseases that occurred later in the growth cycle were classified as root diseases.

Estimates of germling emergence based on approximate number of seed sown are summarized in Table 6 (Appendix). For the USDA Forest Service Nursery, mechanical seeders were calibrated to place two or three seeds per cell, depending on seedlot. As shown in Table 6, many cells had more than three germlings emerge, indicating that more seed were often sown than desired. At the University of Idaho Research Nursery, cells were hand sown with two seeds per cell and actual germling emergence was determined.

Empty cells (without seedlings) sampled at the time of thinning indicated differences among the seedlots tested (Table 7 - Appendix). These differences were significant ($P=0.05$) for several seedlots at the USDA Forest Service Nursery, but not for seedlots at the University of Idaho Research Nursery.

Diseased seedlings 2 or more months old were rated according to a system based on severity of foliar symptoms (Table 8 - Appendix). Root disease severity at the USDA Forest Service and University of Idaho Research Nurseries is summarized in Tables 9 and 10, (Appendix) respectively.

Roots of weeds sampled at the USDA Forest Service Nursery were often infected with *Fusarium* spp., particularly *F. oxysporum* Schlect. Weeds had often been killed by a saline water solution; foliage from weeds killed this way also often yielded *Fusarium* spp.

Asymptomatic Seedling Infection

Infection of asymptomatic seedlings with *Fusarium* spp. is summarized for the USDA Forest Service and University of Idaho Research Nurseries in Tables 11 and 12 (Appendix), respectively. Root colonization of seedlings without disease symptoms was generally higher at the USDA Forest Service Nursery, although all seedlots tested had many infected seedlings. Differences among seedlots were not statistically significant at either nursery; there were also no differences in asymptomatic seedling root infection between seedlots treated with bleach and those not treated at the University of Idaho Research Nursery.

Comparisons using linear regressions among several variables are summarized in Table 13 (Appendix). Comparisons were made to possibly predict disease incidence, asymptomatic seedling infection, and seedling emergence from levels of *Fusarium* on sampled seedlots. Factors showing relatively high coefficients of determination (R) included (1) *Fusarium* on seed vs. asymptomatic seedling infection, (2) disease incidence vs. asymptomatic seedling infection, and (3) root disease severity rating vs. extent of *Fusarium* colonization of roots.

Secondary Disease Spread

No lateral spread of *Fusarium* spp. was detected on the basis of production of root disease symptoms on adjacent seedlings within foci established to monitor secondary spread at the USDA Forest Service Nursery. Even though diseased seedlings in the center of foci were selected because of production of *Fusarium* sporodochia on the above-ground portion of their stems, there was no evidence that the fungi spread to nearby seedlings and caused disease.

DISCUSSION

These investigations confirmed the common occurrence of *Fusarium* on Douglas-fir seed. Levels found approximated those reported previously (James 1984; James 1986b). However, the level of *Fusarium* on seed was an unsatisfactory predictor of disease incidence within a particular seedlot. This may have been due to relatively low levels of disease encountered in this study and because seed assays included all isolates of *Fusarium*, saprophytes as well as parasites. It is likely that many isolates detected on seed were not capable of causing disease even though they might have infected seedling roots. The high correlation between seed infection and asymptomatic seedling root infection would substantiate this conclusion.

At the University of Idaho Research Nursery, good inverse correlation existed between the amount of *Fusarium* and the amount of *Trichoderma* on seed. Seed with low populations of the former generally had high populations of the latter. Since *Trichoderma* spp. are common antagonists of many pathogens, including species of *Fusarium* (Elad et al. 1982; Papavizas 1985), this inverse relationship is understandable. Also, treatment of seed with bleach prior to sowing resulted in greater amounts of *Fusarium* on seed; however, levels of *Trichoderma* on bleach-treated seed were much reduced. Apparently, *Trichoderma* spp. were more susceptible to the bleach treatment than *Fusarium* spp. When *Trichoderma* were reduced or eliminated by the treatment, *Fusarium* could increase because of the reduction of the *Trichoderma* antagonists. Since bleach treatment occurred prior to stratification, *Fusarium* could have possibly spread and increased in treated seedlots during the stratification period. However, the increased level of *Fusarium* on seed did not result in greater amounts of disease in treated seedlots, although asymptomatic seedling root infection was greater in these seedlots.

The other major group of fungi assayed on seed (*Penicillium* spp.) were not related to occurrence of *Fusarium* on seed. This group is not known to be antagonistic toward or highly competitive with *Fusarium*. Levels of these fungi varied widely among the seedlots sampled, and their occurrence on seed may be related to factors of seed collection, processing, and storage.

Occurrence of *Fusarium* as a common colonizer of roots of seedlings without disease symptoms is common (James 1986b; James and Gilligan 1987). There may be certain species or strains of these fungi that more commonly infect roots without eliciting a disease. In certain agricultural crops, isolates of *Fusarium* have been identified that readily infect roots without causing diseases (Armstrong and Armstrong 1948; Chi et al. 1964). In some cases, these saprophytic isolates actually preclude infection by pathogens because they occupy all available infection sites. Such a process is called "cross protection" (Damicone and Manning 1982; Davis 1967). Analyses of the different *Fusarium* isolates obtained from seed and those from diseased and asymptomatic seedlings may help answer our questions regarding pathogenic characteristics of the different isolates. Isolates obtained from seed, diseased seedlings, and asymptomatic seedlings will be analyzed for pathogenicity. Tests are also planned to evaluate fate of asymptomatic seedlings with *Fusarium* root infection after outplanting.

These investigations failed to show spread of *Fusarium* diseases from one container to another. Although only a few plots were established to monitor spread, no disease spread was detected during the 4-month period of monitoring. In most cases, diseased seedlings decayed and became unnoticeable. Although *Fusarium* sporulated initially on diseased seedlings, evidence of this sporulation decreased with time. It is likely that infective spores were disseminated from infected seedlings to nearby healthy seedlings; however, spore movements were not monitored and they may not have reached nearby seedlings in sufficient quantities to cause disease.

The factors influencing occurrence of diseases associated with *Fusarium* spp. in containerized Douglas-fir seedlings are complicated. Interactions of many different organisms and associated environmental conditions are probably necessary for the production of disease. Presence of *Fusarium* in the growing environment is only one of these factors and should not necessarily concern growers. Nonetheless, there are probably levels of inoculum either on seed or within greenhouses that are sufficient to cause serious disease problems (James et al. 1986). Unfortunately, these threshold levels are not easily determined.

Most growers apply fungicides to reduce losses from damping-off during the period of seed germination and seedling emergence. Our investigations indicated that this is the period of greatest disease hazard. Levels of disease subsequent to the damping-off period may not necessarily be of sufficient importance to require chemical treatment, particularly if secondary spread and infection are not important.

Additional work is needed to understand the importance of other sources of *Fusarium* inoculum in container facilities, including the importance of residual inoculum on containers or within greenhouses. Another important area of investigation centers around the interactions between *Fusarium* spp. capable of causing seedling

diseases and other microorganisms in the greenhouse environment. It is likely that these interactions are very important in determining the severity of disease that will occur.

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APPENDIX

Table 1.--Douglas-fir seedlots selected for investigations of *Fusarium* diseases at the USDA Forest Service and University of Idaho Research Nurseries.

USDA Forest Service Nursery, Coeur d'Alene

Seedlot	Origin	Seed/lb.	Germination 1/	Trays sown	Seed/ cell
2682	Fisher River RD Kootenai NF	40,200	76	136	3
2741	Clearwater RD Nezperce NF	37,600	73	37	3
4010	Powell RD Clearwater NF	49,200	78	107	2
4486	Elk City RD Nezperce NF	47,600	75	376	3
6070	Hebgen Lake RD Gallatin NF	38,300	84	275	2

University of Idaho Research Nursery, Moscow

Seedlot	Origin	Seed/lb.	Germination 1/	Trays sown	Seed/ cell
DAW	Sandpoint, Idaho	43,280	92	66	2
FN	Bovill, Idaho	44,000	89	111	2
IDL	St. Maries, Idaho	45,360	91	27	2

1/ Basic germination rates as reported for these seedlots by the Nursery.

Table 2.--Occurrence of *Fusarium*, *Trichoderma*, and *Penicillium* species on Douglas-fir seed from selected seedlots from the USDA Forest Service Nursery, Coeur d'Alene, Idaho and the University of Idaho Research Nursery, Moscow *1/*

Seedlots	Fusarium			Trichoderma	Penicillium
USDA Forest Service Nursery	On seedcoat	Within endo-sperm	On seed debris	On seedcoat	On seedcoat
2682	1.7B 4/	2.0	10.0	91.0A 4/	49.0C 4/
2741	15.0A	2.0	5.0	76.0A	81.0AB
4010	1.3B	2.0	5.0	20.0B	97.0A
4486	5.7AB	8.0	15.0	81.0A	78.0BC
6070	14.7AB	6.0	0	24.0B	76.0BC
All	8.1	4.0	7.0	58.4	76.2
Univ. of Idaho Research Nursery	On seedcoat	Within endosperm		On seedcoat	On seedcoat
DAW-T 2/	6.4BC 4/	2.0		52.8C 4/	46.0D 4/
DAW-U 3/	4.8BC	0		99.2A	17.2E
All DAW	5.6 5/	1.0		76.0 5/	31.6 5/
FN-T	13.6B	0		25.6D	78.8B
FN-U	4.8C	0		80.8B	63.2C
All-FN	9.2 5/	0		53.2 5/	71.0 5/
IDL-T	36.0A	0		6.8E	82.8B
IDL-U	2.8C	0		36.4CD	97.6A
All IDL	19.4 5/	0		21.6 5/	90.2 5/
All - T	18.7	0.6		28.4	69.2
All - U	4.1	0		72.1	59.3
All	11.4 5/	0.3		50.3 5/	64.3 5/

1/ Figures in table represent percentages of seed sampled that were colonized by appropriate fungi.
2/ T = Treated with bleach
3/ U = Untreated
4/ Within each column, means followed by the same capital letter are not statistically different (P = 0.05) using Tukey's comparison test.
5/ Treated vs untreated means compared with standard "t" test:

Significance of "t" test

	Fusarium	Sign	Trichoderma	Sign	Penicillium	Sign
DAW	NS	+	P = 0.01	-	P = 0.01	+
FN	P = 0.01	+	P = 0.01	-	P = 0.01	+
IDL	P = 0.01	+	P = 0.01	-	P = 0.01	-
All Lots	P = 0.01	+	P = 0.01	-	NS	+

Sign: + = Treated greater than untreated; - = Untreated greater than treated.

Table 3.--Germination of selected seedlots from the USDA Forest Service Nursery, Coeur d'Alene, Idaho, and the University of Idaho Research Nursery, Moscow.

USDA Forest Service Nursery

Seedlot	Percent Germination 2/ 4/	Percent Germination 3/
2682	44.7C	76
2741	46.0BC	73
4010	77.6A	78
4486	22.3C	75
6070	76.7AB	84

University of Idaho Research Nursery

Seedlot	Treatment 1/	Percent Germination 2/ 4/
DAW	T	95.0 A
DAW	U	91.7 AB
ALL DAW	-	93.3 5/
FN	T	90.9 AB
FN	U	88.9 B
ALL FN	-	89.9 5/
IDL	T	94.7 A
IDL	U	94.8 A
ALL IDL	-	94.8 5/
ALL	T	94.3 5/
ALL	U	91.8 5/

1/ T = treated with bleach; U = untreated

2/ Germination after 28 days; USDA Forest Service Nursery seedlots germinated on water agar; University of Idaho Research Nursery seedlots germinated on peat-vermiculite soil mix.

3/ As reported by the nursery for standard germination tests.

4/ For each nursery, means followed by the same capital letter are not statistically different (P=0.05) using Tukey's comparison test.

5/ Treated vs. untreated means compared with standard "t" test:

Significance of "t" test

Seedlot	Germination	Sign
DAW	NS	+
FN	P=0.05	+
IDL	NS	+
All lots	P=0.05	+

Sign: + = treated greater than untreated.

Table 4.--Regression equations and coefficients of determination for comparisons among seed-colonizing fungi and germination for the USDA Forest Service Service Nursery, Coeur d'Alene, Idaho, and the University of Idaho Research Nursery, Moscow.

USDA Forest Service Nursery

Comparison 1/	No.	Regression equation 2/	Coefficient of determination
Fusarium vs Germination	1	Y = 50.42 - 0.30 x	0.02
Fusarium (E) 3/ vs Germination	2	Y = 61.29 - 1.94 x	0.27
Trichoderma vs Germination	3	Y = 129.43 - 21.52 log x	0.80
Trichoderma vs Fusarium	4	Y = 10.45 + 0.01 x	0.01
Penicillium vs Trichoderma	5	Y = 145.65 - 1.44 x	0.59

University of Idaho Research Nursery *4/*

Comparison 1/	No.	Regression equation 2/	Coefficient of determination
Fusarium vs Germination	6	Y = 77.20 + 0.11 x	0.13
Trichoderma vs Germination	7	Y = 79.35 - 0.07 x	0.33
Trichoderma vs Fusarium	8	Y = 66.71 - 13.95 log x	0.69
Penicillium vs Trichoderma	9	Y = 83.31 - 0.59 x	0.58

1/ Independent variable (X) listed first and dependent variable (Y) listed second.

2/ All equations represent simple linear regressions unless otherwise noted.

3/ E = Occurrence within seed endosperm.

4/ All comparisons were made using combined bleach-treated and untreated values.

Table 5.--Incidence of disease within selected Douglas-fir seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho *1/*

Sample date	Seedlot 2682	2741	4010	4486	6070	Average disease incidence *3/*
3/86 *2/*	9.0	22.5	2.5	15.8	5.1	11.0 A
4/86	0.5	1.2	1.4	2.9	0.7	1.3 B
5/86	0.1	0.5	0.3	0.5	0.3	0.3 B
6/86	0.1	0.2	0.4	0.2	0.3	0.3 B
7/86	0.0	0.5	0.1	0.5	0.2	0.3 B
9/86	0.0	0.1	0.1	0.0	0.0	0.1 B
Overall *4/*	1.6	4.3	0.8	3.7	1.2	2.3

1/ Values in table represent percentage of cells sampled (1,000 per seedlot) with diseased seedlings.

2/ Sample taken prior to thinning; diseased seedlings included those with damping-off and cotyledon blight. All other samples taken after thinning.

3/ Within the column, all means followed by the same capital letter are not significantly different (P=0.05) using Tukey's Comparison Test.

4/ Mean differences among seedlots not statistically significant (P-0.05).

Table 6.--Emergence of germlings within selected Douglas-fir seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho, and the University of Idaho Research Nursery, Moscow, Idaho.

USDA Forest Service Nursery

Seedlot 1/

No. germlings per cell	2682 No. cells 2/	2741 No. cells	4010 No. cells	4486 No. cells	6070 No. cells
0	22	24	49	118	52
1	11	184	271	285	302
2	385	319	554	309	563
3	356	351	114	224	75
4	108	106	11	56	7
5	17	15	1	6	1
6	1	1	0	1	0
Percent emergence	85.2	82.1	93.2	63.3	84.3
Avg. number germlings per cell	2.5	2.4	1.8	1.8	1.7

University of Idaho Research Nursery

Seedlot 1/

	DAW 2/			FN			IDL		
No. germlings per cell	T 3/	U 4/	All	T	U	All	T	U	All
0	0	0	0	6	7	13	3	0	3
1	46	75	121	75	68	143	47	49	96
2	350	321	671	315	321	636	346	347	693
Percent emergence	94.2	90.5	92.4	89.0	89.6	89.3	93.3	93.8	93.6
Avg. number germlings per cell	1.9	1.8	1.8	1.8	1.8	1/8	1.9	1.9	1.9

1/ USDA Forest Service Nursery: Average number of seed sown per cell 2682, 2741, 4486 = 3; 4010, 6070 = 2. University of Idaho Research

2/ USDA Forest Service Nursery: total number of cells per seedlot = 1,000 (200 per tray for five trays); University of Idaho Research Nursery: total number of cells per seedlot = 792 (198 per tray for two trays per treatment).

3/ Seed treated with bleach prior to sowing.

4/ Seed not treated with bleach prior to sowing.

Table 7.--Estimates of empty cells (without seedlings) within selected Douglas-fir seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho, and the University of Idaho Research Nursery, Moscow.

USDA Forest Service Nursery

	Seedlot				
	2682	2741	4010	4486	6070
No. cells sampled	11,200	5,400	13,200	25,800	20,600
Percent cells without seedlings *1/*	2.7 B	5.2 AB	7.3 A	9.1 A	5.6 AB

University of Idaho Research Nursery

	Seedlot *2/*								
	DAW			FN			IDL		
	T *3/*	U *4/*	All	T	U	All	T	U	All
Percent cells without seedlings *1/*	0.0	0.0	0.0	1.5	1.8	1.6	0.8	0.0	0.4

1/ Within the row, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's comparison test. Mean differences for the University of Idaho Research Nursery not significantly different (P=0.05).

2/ A total of 792 cells sampled per seedlot for the University of Idaho Research Nursery.

3/ Seed treated with bleach prior to sowing.

4/ Seed not treated with bleach prior to sowing.

Table 8.--Description of *Fusarium* root disease severity rating system used at the USDA Forest Service Nursery, Coeur d'Alene, Idaho, and the University of Idaho Research Nursery, Moscow

Numerical rating	Description
0	Seedling crown entirely green.
1	Seedling with slight needle tip dieback, particularly concentrated on the upper whorls.
2	Seedling with lower whorl of needles partially or completely necrotic; seedling upright.
3	Seedling with needle tip dieback affecting at least one-half of the crown.
4	Seedling with necrotic foliage on at least one-half of its crown (upper or lower); seedling upright.
5	Seedling with necrotic foliage on at least one-half of its crown (upper or lower); seedling bent over.
6	Seedling with necrotic foliage on at least three-fourths of its crown; seedling upright or bent over.
7	Seedling with its entire crown necrotic; seedling upright or bent over.

Table 9.--Severity of *Fusarium* root disease within selected Douglas-fir seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho *1/*

Root disease rating 2/	Sample Dates 4/86	5/86	6/86	7/86	9/86	Average for all samples
7	30.0	8.1	40.3	32.4	27.3	26.7
6	32.0	2.7	6.9	10.8	18.2	12.2
5	18.0	10.8	4.2	2.7	4.5	8.6
4	12.0	9.6	6.9	8.1	9.1	9.0
3	0.0	33.8	8.3	10.8	18.2	15.3
2	8.0	18.9	6.9	10.8	13.6	11.8
1	0.0	16.2	26.4	24.3	9.1	16.5
Average rating	5.6	3.2	4.4	4.2	4.5	4.2
Number sampled	50	74	72	37	22	Total = 255
Fusarium colonization 3/	83.0	73.0	72.2	86.5	90.9	78.2
Colonization percent 4/	83.8	38.0	41.5	53.5	48.8	51.5

1/ Values in table represent percent of sampled seedlings with appropriate root disease rating.

2/ See Table 8 for description of root disease ratings.

3/ Percentage of diseased seedlings with roots colonized by Fusarium spp.

4/ For seedlings with roots infested with Fusarium spp., average of their root systems colonized by Fusarium spp.

Table 10.--Severity of *Fusarium* root disease within related Douglas-fir seedlots at the University of Idaho Research Nursery, Moscow *1/*

Root disease rating *2/*	Sample Dates 6/86	7/86	8/86	9/86	Average for all samples
7	16.7	0.0	25.0	42.9	26.5
6	0.0	16.7	0.0	0.0	2.9
5	83.3	16.7	0.0	7.1	20.6
4	0.0	50.0	0.0	7.1	11.8
3	0.0	16.7	62.5	21.4	26.5
2	0.0	0	0.0	7.1	2.9
1	0.0	0	12.5	14.3	8.8
Average rating	5.3	4.3	3.8	4.6	4.5
Number sampled	6	6	8	14	Total = 34
Fusarium colonization *3/*	100.0	65.7	75.0	50.0	67.7
Colonization percent *4/*	71.5	83.3	100.0	98.4	92.3

1/ Values in table represent percent of sampled seedlings with appropriate root disease rating.

2/ See Table 8 for description of root disease ratings.

3/ Percentage of diseased seedlings with roots colonized by Fusarium spp.

4/ For seedlings with roots infected with Fusarium spp., average of their root systems colonized by Fusarium spp.

Table 11.--Colonization of asymptomatic Douglas-fir seedling roots with Fusarium spp. at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Seedlot									
	2682		2741		4010		4486		6070	
Sample date	No.[1/]	Colon.[2/]	No.[1/]	Colon.[2/]	No.[1/]	Colon.[2/]	No.[1/]	Colon.[2/]	No.[1/]	Colon.[2/]
3/86	41.2	16.5	73.7	22.3	16.7	9.3	30.8	26.2	31.6	19.3
4/86	20.0	45.0	100.0	53.0	50.0	10.0	100.0	46.0	90.0	17.2
5/86	60.0	24.2	90.0	33.9	60.0	27.5	90.0	42.8	100.0	26.0
7/86	80.0	22.8	70.0	22.9	60.0	33.3	80.0	31.3	50.0	33.0
Averages[3/]	48.9	20.6	81.6	27.0	41.7	17.9	72.1	36.2	61.2	22.5

1/ Percentages of seedlings sampled with Fusarium spp. colonizing their roots.

2/ Average percentage colonization of roots with Fusarium spp. (infected seedlings only).

3/ Differences among seedlots and sampling dates not statistically significant (P=0.05) using an analysis of variance.

Table 12.--Colonization of asymptomatic Douglas-fir seedling roots with *Fusarium* spp. at the University of Idaho Research Nursery, Moscow

Seedlot	Sampling Dates: 5/86 No.[1]	5/86 Colon.[2]	6/86 No.[1]	6/86 Colon.[2]	7/86 No.[1]	7/86 Colon.[2]	9/85 No.[1]	9/85 Colon.[2]	1/87 No.[1]	1/87 Colon.[2]	Averages No.[1]	Averages Colon.[2]
DAW-T[3]	8.3	-	60.0	28.6	50.0	55.0	30.0	57.1	40.0	18.8	31.3	31.9
DAW-U[4]	11.5	-	20.0	38.5	20.0	62.5	20.0	33.3	50.0	17.0	21.2	23.7
All DAW	10.0	-	40.0	31.3	35.0	57.1	25.0	47.8	45.0	17.8	26.1	28.1
FN-T	11.1	-	50.0	71.4	63.6	46.7	28.6	42.5	40.0	10.0	29.6	33.1
FN-U	8.3	-	50.0	39.1	30.8	73.3	36.4	86.7	30.0	10.0	23.8	38.3
All FN	9.7	-	50.0	56.9	45.8	55.5	32.0	78.9	35.0	10.0	26.7	35.6
IDL-T	25.0	-	70.0	34.9	60.0	40.7	60.0	33.3	60.0	10.0	48.4	24.1
IDL-U	4.2	-	40.0	25.0	70.0	33.3	10.0	20.0	60.0	13.3	29.7	18.5
All IDL	14.6	-	55.0	31.3	65.0	36.8	35.0	31.3	60.0	11.8	39.1	21.6
All T[5]	14.3	-	60.0	42.4	58.1	46.7	38.2	41.8	46.7	12.7	37.8	29.2
All U[5]	8.1	-	36.7	33.3	39.4	49.1	22.6	53.1	46.7	13.9	24.8	26.0
All seedlings[6]	11.2	-	48.3	39.2	48.4	47.7	30.8	45.7	46.7	13.3	31.3	27.8

[1] Percentage of seedlings sampled with *Fusarium* spp. colonizing their roots.

[2] Average percentage colonization of roots with *Fusarium* spp. (infected seedlings only).

[3] Seed treated with bleach.

[4] Seed untreated.

[5] Mean differences between treated and untreated seedlots not statistically significant (P=0.05) using a standard "t" test.

[6] Differences among seedlots and treatments not statistically different (P=0.05) using a two-way analysis of variance.

Table 13.--Regression coefficients of determination (R^2) for comparisons among amount of seed colonized by *Fusarium*, seedling emergence, disease incidence, empty cell incidence, asymptomatic seedling infection, root disease severity ratings, and root colonization by *Fusarium* for the USDA Forest Service Nursery, Coeur d'Alene, Idaho, and the University of Idaho Research Nursery, Moscow.

Comparison 1/	Coefficient of Determination (R^2)	
	USDA Forest Service Nursery	University of Idaho Research Nursery 2/
Fusarium on seed vs emergence	0.11	0.01
Fusarium on seed vs empty cells	0.01	0.18
Fusarium on seed vs disease incidence	0.50	-- 3/
Fusarium on seed vs asymptomatic infection	0.83	0.76
Emergence vs disease incidence	0.44	-- 3/
Empty cells vs disease incidence	0.19	-- 3/
Disease incidence vs asymptomatic infection	0.72	-- 3/
Root disease severity rating vs root colonization	0.76	0.61

1/ Independent variable (X) listed first and dependent variable (Y) listed second.

2/ All comparisons were made using combined bleach-treated and untreated values.

3/ Not available because of very low disease incidence.